"Some Observations on the Chemistry of the Contents of the Alimentary Tract under various conditions; and on the Influence of the Bacteria present in them."* By A. Lockhart Gillespie, M.D., F.R.C.P. (Ed.), F.R.S.E. Communicated by Professor J. G. McKendrick, F.R.S. Received May 18—Read June 17, 1897.

(Abstract.)

In this paper are given details of experiments which were designed for the purpose of investigating the grosser chemical changes which occur in the intestinal tract, and the nature and influence of the micro-organisms present in the contents after the ingestion of different kinds of food or after the administration of certain drugs.

The points taken up comprise:—

The reaction of the contents of the alimentary tract in its various parts, and the bodies to which this reaction is due.

The amount of chlorine present, and the nature of its combinations.

The solids present in the contents.

The action of the ferments.

The number and nature of the bacteria.

A series of experiments were first carried out on a dog with an ileac fistula, situated at the ileo-cæcal valve. As these experiments did not prove altogether satisfactory, a further series were made on dogs fed for some days on special diets, or given certain drugs, and killed about three hours after their last meal. After removal of the entire alimentary tract, it was divided into six parts, viz., stomach, duodenum, jejunum, upper ileum, lower ileum, and large intestine, double ligatures having been placed between each section. Inoculations were made from these segments with due antiseptic precautions. Thereafter the contents of each section was analysed.

A similar experiment was carried out on a calf, and another on the contents of a portion of the intestines of a man.

The effects of the following diets were investigated:—

On dogs: 1. *Ordinary*, porridge and milk and some meat.
2. Porridge and milk.
3. Boiled beef.
4. Sterilised milk.

* Towards the expenses of this Research, which was undertaken at the Research Laboratory of the Royal College of Physicians, Edinburgh, a Grant from the British Medical Association was received.

In the calf: Cow's milk. (The calf had not passed the sucking stage.)

The drugs used were: 1. Acidum hydrochloricum dilutum.
2. Sodii carbonas.
3. Salol.
4. Calomel.
5. Creasote.
6. Benzosol.
7. Guaiacol carbonas.
8. Ammonii chloridum.

In each instance, two meals were given in the day, and when drugs were used two doses in the twenty-four hours, administered with each meal.

The contents of the ileum were obtained each morning from the dog with the fistula by means of a bag tied over the opening.

The Reaction of the Contents.

1. *Total Acidity.*—The total acidity was estimated in the usual way. In the majority of the observations phenol-phthalein was used as an indicator, with check estimations performed with litmus.

In no instance were the contents of any part of the alimentary canal found to be alkaline.

The total acidity of the contents of the duodenum was almost always above that of the gastric contents, while the acidity present in the large intestine was usually as great as, and ofter greater than, that of the duodenal contents.

On an average the maximum acidity occurred in these sections of the tract, the minimum in the contents of the lower half of the ileum.

A curve formed from the mean values for the acidities in the different sections rises from the figure for the stomach contents to that for the duodenal section, gradually falls until the large intestine is reached, when it rises again abruptly.

The greater the proportion of proteids in the food the higher was the acidity of the stomach and duodenal contents, while the material present in the lower parts of the small intestine was less acid, and the contents of the large bowel of rather higher acidity.

The administration of acid with the food increased the acidity of the contents of the duodenum and large intestine, of alkali decreased it in the stomach, but increased it in the other sections. Compared with the results obtained after the administration of the acid, the alkali caused a marked diminution of acidity in the stomach, but an increased acidity in the small intestine below the duodenum.

Salol differed in its effects from calomel in that it caused no

diminution of the acidity throughout the tract, while calomel was followed by a marked fall in the acidity values obtained from the contents of every section, but especially of the small intestine. Guaiacol carbonate and benzosol increased the acidity of the contents of the ileum in the dog with the fistula.

2. *Acidity after Evaporation at* 100° *C. and Acidity driven off.*—The acidity remaining after drying the fluids at 100° C. was least in the contents of the stomach, most in those of the duodenum when the mean figures for all the observations were analysed. With the exception of the stomach and large intestine the acidities, after evaporation, exceeded those of the original samples. That is to say, more alkali was driven off than acid. This in all probability was due to the breaking up of ammonium lactate by heat and the volatilisation of the ammonia. In all cases the discrepancy between the acidity values obtained with phenol-phthalein and those with litmus varied directly with the difference between the values before and after evaporation. If the acidity was less in amount after evaporation than before, the results as shown by the two indicators coincided; if greater, the value as shown by litmus was less than that arrived at by the use of phenol-phthalein.

This increase of acidity after drying was least after food containing much proteid material. After hydrochloric acid it was marked, and after sodium carbonate very slight. The administration of salol was followed by the presence of free acid throughout the tract, that of calomel by an excess of volatile alkali over volatile acid. Of the other antiseptics tested, creasote acted in a similar manner to calomel but to a less extent, guaiacol and benzosol like salol.

The Acid Factors Present.

In the stomach the acidity was due to hydrochloric acid, either free or combined with proteids, and to a small extent to acid salts. In the duodenum the acidity was caused to a great extent by the presence of hydrochloric acid combined with proteids expelled from the stomach, but also to organic acids, such as acetic and lactic acids. In the lower sections of the tract these organic acids were present along with acid salts, and smaller quantities of other acids belonging to the same series.

Chlorine.—The chlorine contained in the intestinal contents was separated into total chlorine, chlorine driven off by evaporation at 100° C. and chlorine not so volatilised, and which was further separated into a part burnt off at a low red heat, and a part remaining after incineration.

The total proportion of chlorine in the different sections showed a slight decrease from the stomach down to the upper half of the ileum,

and then an increase. This increase in the two lower portions was due to the inorganic chlorides. The chlorine, in combination with organic bodies was found in greatest quantity in the stomach, where it was combined for the most part with proteids. It gradually became less in amount in the intestines, except in the lower ileum, here, however, one observation, in which the contents of that section were very concentrated, caused its average proportion to rise to a high level. Volatile chlorine was detected in the stomach contents, in the duodenum, and other parts of the small intestine. None was found in the large bowel. In the stomach and duodenum much of it was in the form of hydrochloric acid, below the duodenum it was probably present as unstable compounds of ammonia, or of other organic bases formed from proteids by the action of trypsin.

In those instances where the acidity of the stomach contents was high, as after proteid food, the chlorine present in the bowel was present in larger quantity than the average.

Solids.—The total solids at 110° C. were in the largest proportion in the lower part of the ileum. Unfortunately, owing to the large intestine having been emptied shortly before the time when many of the analyses were made, the values obtained for the contents of this section of the canal must be regarded as fallacious. The solids were in lowest proportion in the large intestine, and only slightly greater in the stomach. A much higher figure was obtained from the contents of the duodenum than from the stomach.

The inorganic ash was present in larger proportion in the duodenum, jejunum, and lower ileum than in the other sections.

Bacteriology.

The organisms grown from the contents of the six sections into which the alimentary canal was divided were found to vary in number and character with the degree of acidity present. The higher the acidity the greater was the proportion of acid-forming growths, the majority of which were unable to liquefy gelatine, and, as a rule, the smaller was the total number of bacteria cultivated.

When the acidity was low a greater number of liquefying organisms could be grown, most of which rendered the nutrient medium alkaline in reaction.

When an ordinary diet (porridge, milk, and some meat) was given, the contents of the duodenum yielded the fewest colonies, those of the stomach a few more, while below the duodenum the numbers grown increased progressively. A purely meat diet caused an almost entire disappearance of growths from the tubes inoculated from the stomach, duodenum, and jejunum, a marked fall below the average in the numbers in the ileum, although the colonies obtained from the large intestine were as numerous as usual.

No growths were obtained from the contents of the stomach or jejunum of a dog fed on sterilised milk, and only four from the material in the duodenum. Large numbers were present below this part. The contents of the entire intestinal canal of a calf did not yield a single liquefying organism, while the total number grown was very small, as far down as the lower half of the ileum.

The administration of dilute hydrochloric acid was followed by a decrease in the number of organisms present in each section of the canal, and especially of the organisms capable of liquefying gelatine. Carbonate of sodium, on the other hand, caused no diminution in the numbers found in the upper parts of the tract, but—corresponding to an increase in acidity in the contents of the lower sections—occasioned a diminution of liquefying forms in the ileum and large intestine.

The administration of antiseptic drugs yielded very interesting results. When salol was given, no diminution in the number of organisms was observed until the ileum was reached, when the organisms capable of liquefying gelatine became very few in number. After calomel, the upper sections contained a small number of bacteria, the lower parts a large number which were chiefly of the class able to liquefy gelatine.

No trace of the decomposition products of salol could be detected in any of the sections above the upper half of the ileum. In this portion of the canal only a faint reaction was obtained. In the lower part of the ileum and in the large intestine the reaction was well marked.

The exhibition of other antiseptic drugs was quickly followed by a sensible diminution in the number of the organisms present.

Extract of hæmatoxylin was given for the purpose of testing the action of an astringent on intestinal fermentation. The number of organisms was rather increased during its administration.

Trypsin.

Although the contents of the bowel were always acid, trypsin was found to be active in them. Perhaps this fact may explain why this ferment is secreted for so many hours after food, when pepsin is only secreted during the duration of stay of food in the stomach. Acids, however weak, gradually destroy trypsin; the acid in the bowel must do so, but this destructive action is compensated for by a constant secretion of more of the ferment.

The action of trypsin on proteids in the presence of organic acids was investigated in the dog fed upon sterilised milk.

To 10 cub. cm. of a solution of egg-albumin containing 0·115 gram albumin, 5 cub. cm. of the contents of each section of the

alimentary canal were added. This mixture was rendered alkaline with sodium carbonate, and then just made acid by the cautious addition of acetic acid until the neutral point was passed. The mixtures were then kept at 38° C. for four hours. A check experiment with liquor pancreaticus in place of the contents showed that it could digest 68·5 per cent. of the albumin in a slightly acid solution. The amounts digested by the intestinal contents varied from 48 per cent. in the case of the lower ileum, 37 per cent. in the jejunum, to 17 per cent. in the large intestine.

A small proportion of free mineral acid arrested the proteolytic action of trypsin; a larger percentage of hydrochloric acid combined with proteids was necessary to cause a corresponding degree of inhibition.

Pepsin.

Artificial digestion experiments, in which 5 cub. cm. of the contents of each portion of the alimentary canal were added to a mixture containing 0·115 gram of egg albumin and 20 cub. cm. of decinormal HCl, resulted in a certain amount of the albumin being digested in each. Seventy-seven per cent. of the proteid was digested after four hours at 38° C., when 5 cub. cm. of the stomach contents had been added, 20 to 28 per cent. in the experiments with the duodenal, jejunal, and upper ileac contents, only 12·9 per cent. when the contents of the lower ileum were used, and 19 per cent. when the large intestine was tested.

Distillate of Contents of Bowel.

When the contents of a portion of the intestine were distilled after the addition of water, if the combined acidity after evaporation was greater than the total acidity as originally estimated, the first portions of the distillate were alkaline and contained ammonia, even although the contents had been of highly acid reaction. When the acidity after evaporation was lower than before it, as in the stomach and large intestine, the distillate was acid from the first, due, as a rule, to acetic acid. In most cases the acidity of the residue was found to be chiefly composed of lactic acid.

Proteids.—Coagulable proteids were obtained from the contents of the lower ileum from the dog with a fistula on each occasion, varying from 1·92 per cent. to a mere trace. No relation could be traced between the other factors and the quantity of coagulable proteid present.

On another occasion half-saturation of the contents with ammonium sulphate, which precipitates globulins, brought down much more than the half of the total coagulable proteid present, except in the contents of the stomach. Albumoses were found in the

stomach in large amount, none in the duodenum or jejunum, and traces in the lower sections of the bowel. Peptones were present in traces in the contents of the stomach and jejunum and in the parts below it.

General Conclusions.

1. The contents of the intestinal canal in the dog and calf, and probably in man, are acid in reaction throughout; the acidity being due to organic acids formed by micro-organisms, hydrochloric acid in combination with proteids and proteid derivatives, and to acid salts.

2. When the food passes from the stomach into the duodenum it rapidly becomes more concentrated from absorption of water, and consequently more acid; it still contains a large proportion of hydrochloric acid in combination with proteid bodies, but the increased proportion of inorganic chlorides indicates that this acid is rapidly being acted on by the soda of the pancreatic secretion.

3. The organisms present in the bowel are divisible into two great groups, those which are able to give the medium in which they grow an acid reaction, and those which cause it to become alkaline or neutral. The first class, as a class, are usually unable to liquefy gelatine. The second class can do so, and form the ordinary putrefactive organisms. These classes are mutually antagonistic. If the number of acid-forming organisms be in large proportion to the total number present, the second class of bacteria fails to grow in any luxuriance, and the intestinal contents do not putrefy in the ordinary sense of the word. *Per contra*, should the liquefying and alkali-forming organisms be in the majority, the first class is less numerous, and intestinal putrefaction may be present. As, however, the diminished acidity which follows the growth and action of the second class of bacteria is favourable for the multiplication of members of the first class, sufficient acid is formed by them in ordinary cases to neutralise the alkali, and generally to cause the reaction to remain acid. The ammonia formed by the second class often unites with the lactic acid produced by the first, creating, in fact, a salt which is very advantageous for the further development of both classes.

4. A normal acidity of the stomach contents with the presence of free HCl, or an increased amount of each of these factors, causes a greater destruction of the alkali-forming or putrefactive bacteria than of the acid-forming and more resistant organisms. This naturally leads to diminished decomposition in the bowel. A diminished gastric acidity, or a large meal chiefly proteid in character, allows a larger number of the second class of bacteria to reach the bowel, and may thus cause intestinal decomposition and indigestion. But such a result is not invariable, as the diminished

acidity of the upper parts of the canal favours, in health at least, the growth of the acid-forming bacteria, and may thus lead to an increased acidity and diminished decomposition in the lower parts of the canal.

5. Some antiseptic substances appear to act more on the first class of organisms than on the second. Thus salol seemed to act more energetically on the liquefying forms than on the acid-forming class, calomel the converse; while salol exerted a greater antiseptic power in the lower part of the intestinal canal, calomel in the upper portions.

6. Trypsin is capable of energetic proteolytic action in the presence of organic acids, but, as it is slowly destroyed by these acids, it has to be constantly supplied in fresh quantities.

7. The figures obtained for the total solids of the different sections show that absorption of fluids is greatest in the duodenum and lower ileum. The absorption from the large intestine can not be compared with the absorption from the other parts owing to the number of times its contents represented the material newly passed from the ileum.

"On a Discontinuous Variation occurring in *Biscutella lævigata*."

By E. R. SAUNDERS, Lecturer of Newnham College, Cambridge. Communicated by W. BATESON, F.R.S. Received June 9,—Read June 17, 1897.

The observations recorded in this paper were made upon *Biscutella lævigata*, a cruciferous plant occurring as a perennial herb in the alpine and sub-alpine regions of middle and southern Europe. It was observed by Mr. Bateson that in a valley of the Italian Alps this species exhibits two distinct forms,* which exist side by side, the one hairy and the other glabrous. Plants showing various degrees of hairiness, and constituting a series of intermediate forms connecting the two extremes, were also found, but were comparatively scarce. As it may be presumed that in the state of nature the two varieties intercross freely, the question arises—how is their distinctness maintained? For on the supposition that hairiness and smoothness are characters capable of blending freely, it might be expected that offspring derived from a cross between hairy and smooth parents would tend constantly to regress to a mean condition of texture. It was in order to test the validity of this supposition, and to ascer-

* Mr. Bateson's attention was drawn to the variations of this species whilst staying in the Val Formazza, for the purpose of studying the alpine forms of the butterfly *Pieris napi*, for it is upon these plants that the variety *bryoniæ* chiefly lays its eggs in this locality.